The time memory of the Venus flytrap (*Dionaea muscipula* Ellis)

Arne Molis • Gabriele Patten • Manfred Weidner • Universität Köln • Botanisches Institut • Gyrhofsstrasse 15 • 50923 Köln • Germany •manfred.weidner@uni-koeln.de

Keywords: physiology: *Dionaea muscipula.*

Summary

For closure to occur, the trap leaves of the carnivorous Venus flytrap (*Dionaea muscipula* Ellis) normally must be stimulated twice. This is achieved by touching the bending-sensitive hairs protruding from the upper leaf surface. This triggers a rapid thigmonastic capture movement of the leaf lobes. This way incidental closure movements are avoided. The maximal memory time, i.e. the time limit between the first and the second stimulation event, which must not be exceeded, is highly variable and ranges between 20—80 s. Our investigation explores the influences of external (environmental) and internal (plant specific) factors on this memory time. Throughout in this study, a "50%-closure-value" was determined for each variable on basis of 50—100 independent measurements. The memory effect exhibits inverse and sigmoidal temperature characteristics, thus showing a maximum memory time at lower temperatures (15°C). Moreover, the daily photon fluency, the age of the trap leaves, seasonality (the time of leaf emergence), and the recovery time between consecutive stimulation cycles, all affect the time memory strongly. Further, the topology of stimulation also exerts a large influence: The time memory fades more rapidly when two different trigger hairs were stimulated successively within the time limits of the memory instead of touching the same hair twice.

Introduction

The evocation of trap leaf closure in the Venus flytrap, *D. muscipula* Ellis, is a unique phenomenon in nature, because this movement normally occurs only after two successive mechanical stimulations of the 3 (-5) special hairs. These 3 to 5 trigger hairs, acting as mechano-sensors, are protruding from the abaxial surface of the two trap lobes (Haberlandt 1906; Guttenberg von 1925; Williams and Mozinga 1971; Juniper *et al.* 1989).This unparalleled behaviour was characterised as manifestation of a "time memory" (Brown & Sharp 1910; Jacobson 1965). We adopt this term but do not intend to imply any explanation for the mechanisms underlying the phenomenon. Evidently, the information of an initial stimulation event is "stored" for a period of about one minute. During this time-span, the leaf is in a preconditioned state for subsequent closure and thus susceptible to a second stimulation; it closes extremely fast upon re-stimulation. Occasionally, more than two stimulations may be required, or closure may be triggered by just one triggering event (Brown 1916; Jacobson 1965; Sibaoka 1966; Williams 1973; Hodick & Sievers 1986, 1988, 1989; Trebacz 1996). However, no systematic and comprehensive description of the phenomenon has ever been undertaken. It was our intention to lessen this gap and evaluate some prominent phenological aspects of the time memory of *D. muscipula*, striving for elucidation of the influence of external as well as internal factors.

A highly condensed recapitulation of the present stage of our knowledge on trap leaf closure with special regard to the time memory of *D. muscipula* is helpful to understand the experimental facts presented and discussed below. Ion fluxes, involving Ca^{2+}, K^+ and Cl^-, cause rapid changes of the cell membrane potential of leaf parenchyma cells, and thus are responsible for the triggering of trap closure. Depending on stimulus-strength, the mechanical stimulation is transduced into a receptor potential, generated in specialised mechano-sensitive cells at the base of a trigger hair (Haberlandt 1906; Benolken & Jacobson 1970; Williams & Mozingo 1971; Casser *et al.* 1985; Hodick & Sievers 1988). If the excitation is strong enough, the receptor potential triggers an action potential ("AP"), which—as a circular depolarisation wave of the cell membrane potential—spreads rapidly across the lobes of the trap leaf (Burden-Sanderson & Page 1876; Williams & Pickard 1980; Hodick & Sievers 1986, 1988; Sibaoka 1966, 1991; Trebacz *et al.* 1996; Volkov *et al.* 2002). Upon arrival of a second AP—evoked within the limits of the memory time—epidermal

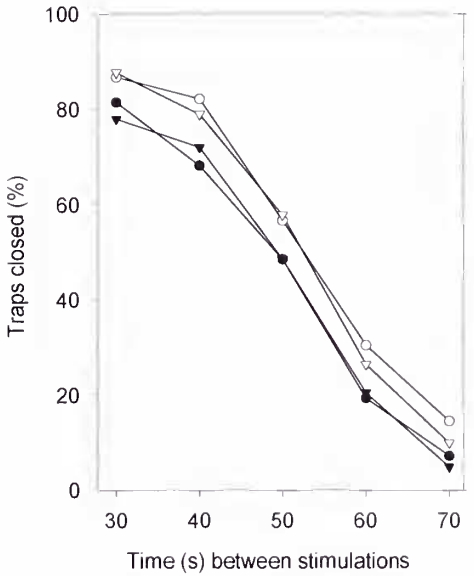

Figure 1: Comparison of traps responding to two stimulations with complete closures (data shown with open circles and open triangles), and traps that responded with either complete or partial closure (i.e. "all closures"; data shown with filled circles and filled triangles). Two series of measurements were executed, one is indicated with circles, the other with triangles.

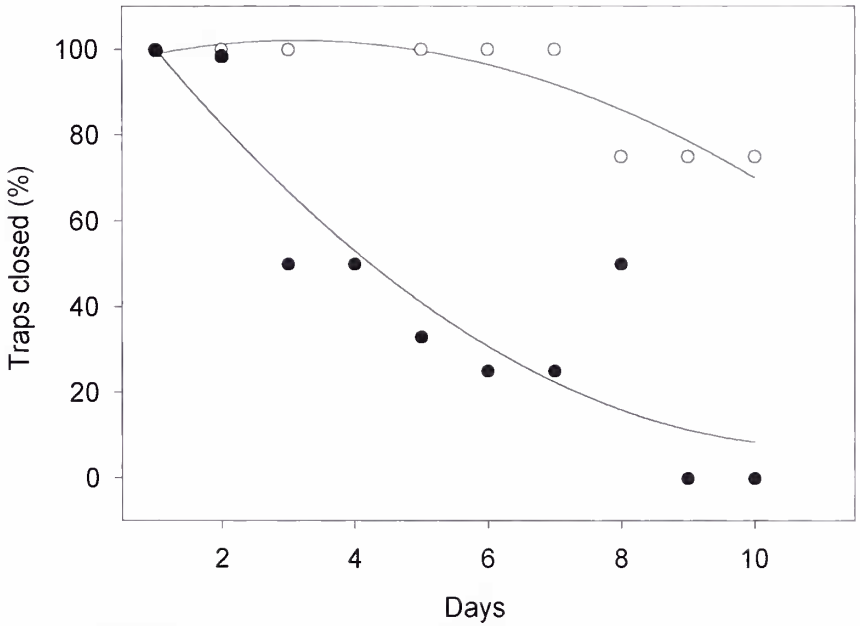

Figure 2: Stimulation repetition experiment: Relatively young trap leaves (3 weeks after emergence) were subjected to 10 stimulation/closure/re-opening cycles. The time lapse between cycles was 24 hours. The interval between first and second stimulation was set at 20 s, which ensured 100% closure at the beginning of the experiment (cycle 1). Complete closures (filled circles) and all closures (open circles) were compared.

and mesophyll cells which are anatomically and physiologically equipped to undergo small changes in cell shape (Guttenberg 1926; Benolken & Jacobson 1970; Haddock & Sievers 1989; Juniper *et al.* 1989) initiate the extremely rapid closure movement of the trap leaf by means of a "snap-buckle" mechanism, as revealed only recently by Fortiori *et al.* (2005).

A breakthrough of equal rank in understanding the second enigmatic feature of *D. muscipula* besides the extreme velocity of movement, the time memory, has not occurred yet. It has been suggested that an abrupt increase of the cytosolic Ca^{2+}-concentration ("$[Ca^{2+}]_{cyt}$"), brought about by stretch-activated Ca^{2+} influx-channels located in the plasmalemma, does not only evoke the generation of receptor potentials and of subsequent APs, (Cosgrove & Hedrich 1991; Wayne 1994; Malone 1996; Trebacz *et al.* 1996), but is as well decisive for the establishment of the time memory. A critical $[Ca^{2+}]_{cyt}$ threshold concentration must be attained or surpassed in epidermal and mesophyll cells engaged in trap closure in order to trigger the closure movement (Hodick & Sievers 1986, 1988; Trebacz *et al.* 1996). Assumedly, this critical level is reached only after the generation of a second AP, which causes a second Ca^{2+} influx-wave, superimposing the first one which not yet has levelled off. Possibly this occurs through the activation of plasma membrane depolarisation-activated Ca^{2+} channels (DACCs) by the APs. ATP-fuelled Ca^{2+}-pumps are thought to counteract this induced increase in $[Ca^{2+}]_{cyt}$ by continuously shuffling calcium from the cytosol back into the calcium storage pools, i.e. into the apoplast, and probably also into the cisternae of the endoplasmatic reticulum (ER) of the cell (Harper 2001; Hetherington & Brownlee 2004). If the second threshold-breaking AP is not generated in time, the $[Ca^{2+}]_{cyt}$ declines to a level too low for the second Ca^{2+}-inburst to push $[Ca^{2+}]_{cyt}$ beyond the threshold. In this case, the memory time is exceeded and trap closure does not occur.

Our new data on the influences of internal and external factors on the time memory of *D. muscipula* should be useful when the cell biological and molecular aspects of the time memory—in part still highly hypothetical—are reconsidered. Moreover, any future investigations may take advantage of the design and standardization of the experiments in our study.

Materials and Methods

Flytrap plants (*Dionaea muscipula* Ellis) were obtained in May 2001 from "Gartenbau Weilbrenner", 67251 Freinsheim (Germany). The potted plants (8 cm diam.) were grown in plastic trays containing 1-2 cm of natural rain water, free of HCO_3^-. They were cultivated in a greenhouse under natural light. The plants were initially sprayed with Bulldock® (Bayer AG, Germany) insecticide, and subsequently protected from *Sciaridae* re-infestation by screen enclosures and sticky yellow pads (Gelbtafeln). These precautions minimised undesirable closing of the trap leaves.

All experiments were executed between mid-May and August 2001. Thereafter, senescence of the trap-leaves (as well as flowering) became a problem. It seemed important from the beginning to be aware of the seasonality of trap-leaf development (Roberts & Oosting 1958). Therefore, date of emergence and morphometric leaf parameters that were suspected to exhibit seasonal differences (i.e. trap size and shape, petiole-length, and leaf colour) were carefully recorded for each plant, because they could possibly influence the time memory. The leaves of all the plants were marked and catalogued with a thin, water-resistant marker pen within one week after appearance, so a detailed time record was established enabling us to select uniform plants and trap leaves for each experiment.

Several days before the experiments were started, the plants chosen were transferred into a growth chamber; conditions were: 25°C, RH 60%, and continuous fluorescent white light (Osram L 58W/25; photon fluency density 110-150 µmol m^{-2} s^{-1}) 24 hours a day. Once again, sticky yellow pads were employed to capture flying insects. Each trap leaf was used for up to three thigmonastic experiments, allowing for a two-day recovery period between experiments. Thereafter the plants were brought back to the greenhouse for at least two weeks before they became candidates for another series of measurements.

Uniform batches of 10 and 30 *D. muscipula* plants, chosen by visual inspection, were included in one experiment, selecting 2-3 trap leaves from each plant. Thus, one set of data, i.e. one statistical mean, was made from at least 30 individual measurements. All recordings of the time memory were taken between 9 and 12. a.m.

The trigger hairs were stimulated by hand with the tip of a paper clip. Unless stated otherwise, the same trigger hair was used for the first and the second stimulation. The second triggering was

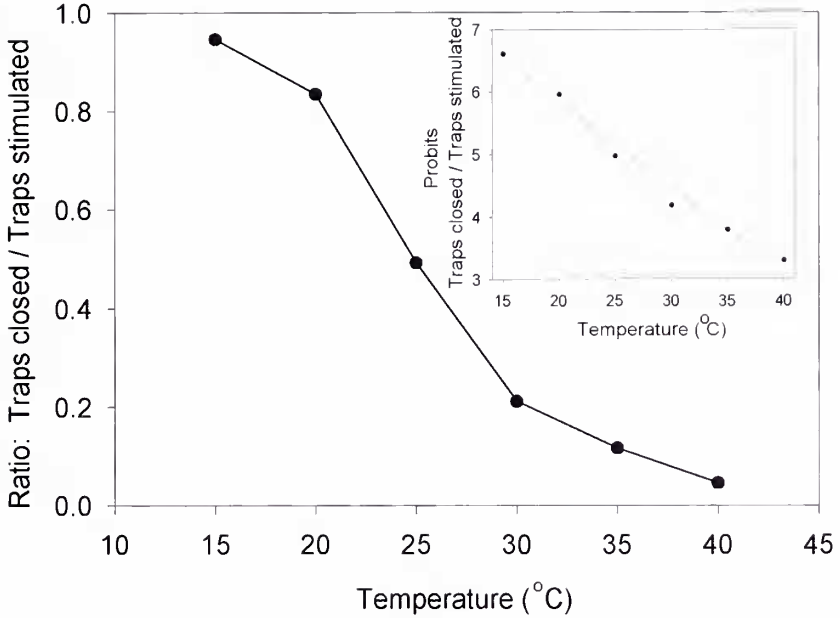

Figure 3: Temperature curve of the time memory of *D. muscipula*. The sigmoidal time memory decreases with rising temperature. The time interval between first and second stimulations was set at 60 s; under these conditions, the traps showed 50% closure at 25°C. The insert shows a Probit transformation of the above data.

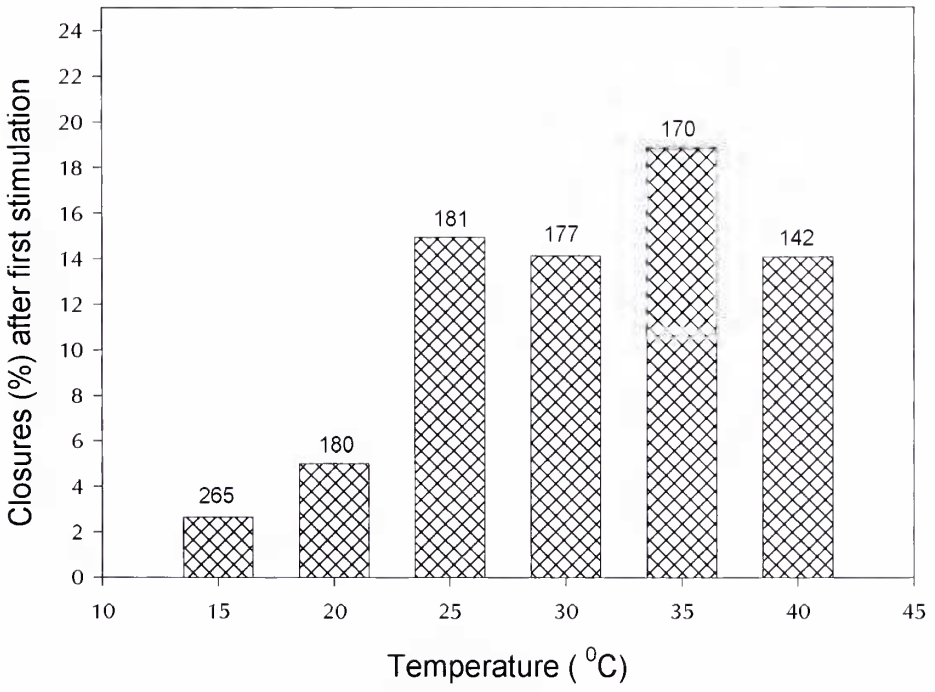

Figure 4: Percentage of closures induced after only one stimulation as a function of temperature. Included are the numbers of traps investigated at each temperature.

executed at variable times after the first one, depending on the particular experimental design. The response of the trap leaf was recorded as either "no closure", "complete closure" (tooth edges of the leaf lobes interlocking), or "incomplete closure" (which includes all responses short of total closure). By merging the values for "complete closures" and "incomplete closures" the category "all closures" was obtained. The time memory was determined by varying the time-lapse between the two consecutive stimulations in the range of 10—80 s in steps of 5—10 s. The percentage of responding traps, expressed either as "complete closures" or "all closures" were fitted into a curve with the time-lapse between stimulations plotted on the abscissa. This way, a curve was obtained that displayed a dose-effect for the memory time. From these plots, the time interval between stimulations for the 50% response was interpolated. This parameter was taken as our quantification of the memory-effect and as such was the basis for characterizing the effects of the external and internal factors on the time memory. In some experiments, e.g. the temperature curve, only one pre-tested time interval was employed.

Incomplete and Complete Trap Closure

It is important to define what is meant by a closure response by the trap. Is it important in our study that a trap leaf responds with complete closure, where the leaf margins tightly interlock or should all touch-induced trap movements, including the incomplete ones, be rated as positive responses?

We compared the time memory of trap stimulations that resulted in complete closures with the time memory of all trap stimulations that resulted in closure (both complete and incomplete). We observed only a moderate difference in the time memory between the two modes of recording (see Figure 1); both, shape and slope of the two response curves were identical. Therefore we conclude that it is insignificant whether the time memory data are compared on the basis of all closures or only complete closures. We used data from all closures in most cases, assuming that the partial closing of a trap leaf cannot be attributed in a causal way to the time memory mechanism itself. In fact, the decidedly parallel course of the two curves suggests that trigger hair stimulation and signal transduction on one side and the mechanical aspect of the closure movement of individual traps on the other side are separate elements of the closure mechanism as a whole. Evidently, only the latter aspect is affected when incomplete closure takes place.

The next experiment takes advantage of the ability of *D. muscipula* trap leaves to reopen repeatedly after an unsuccessful capture movements (Stuhlmann 1948; Jaffe 1973). A trap leaf can be stimulated to close up to 10 or more times, if a recovery time of 24 hours between stimulations was provided. However, upon such repeated stimuli, the percentage of stimuli that resulted in complete closure decreased rapidly (see Figure 2). By the fifth stimulation cycle, only 50% of the traps responded with a complete closure. After 9 repetitions, complete closures no longer occurred. Since the number of times a trap was previously stimulated to close does not affect the trap's time memory, but does modify how the trap is likely to close, this strengthens the view that the time memory and the closure process *in sensu strictu* are distinctly separate aspects of the trap leaf movement. Furthermore, it indicates that each closure movement negatively and quite enduringly affects the physiological state of the trap leaf engaged in the closure process. In other words, the cells and tissues engaged in the closure mechanism are more sensitive—in a metabolic and energetic sense—to optimal conditions than the cells and tissues involved in the time memory. The rapid decline of the ability of the trap to respond with complete closure suggests that those critical cell physiological conditions were not fully re-established, even though the re-opening movement of the incompletely closed traps was unhampered.

Influence of Temperature on the Time Memory

The first environmental factor to be investigated was temperature. In this test, the second stimulation of the trap leaves was executed 60 s after the first one. This setting was found appropriate for the trap leaves chosen for this experiment. At each temperature ≥ 60 traps were tested. The resulting curve exhibits a markedly inverse relationship (see Figure 3) between temperature and memory time. When temperature was increased from 20 to 30°C, the percentage of closures dropped from 84% to 21%, which corresponds to a Q_{10}-value of about 4 for the medium temperature range. The negative correlation suggests that the pronounced prolongation of the memory time,

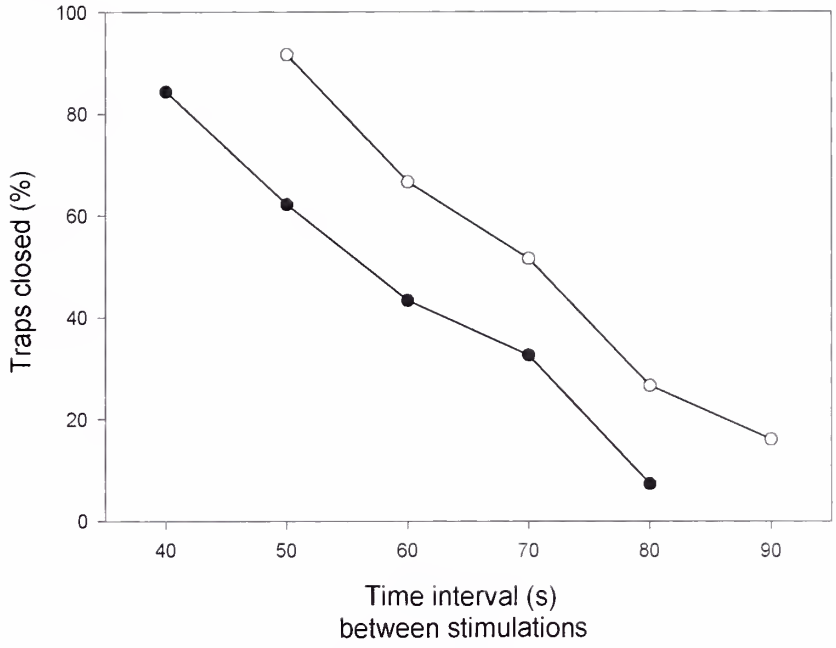

Figure 5: Influence of the daily light period on the time memory. Short-day conditions (8 hours light/16 hours darkness; filled circles) and continuous irradiation (24 hours light/0 hours darkness; open circles) were compared.

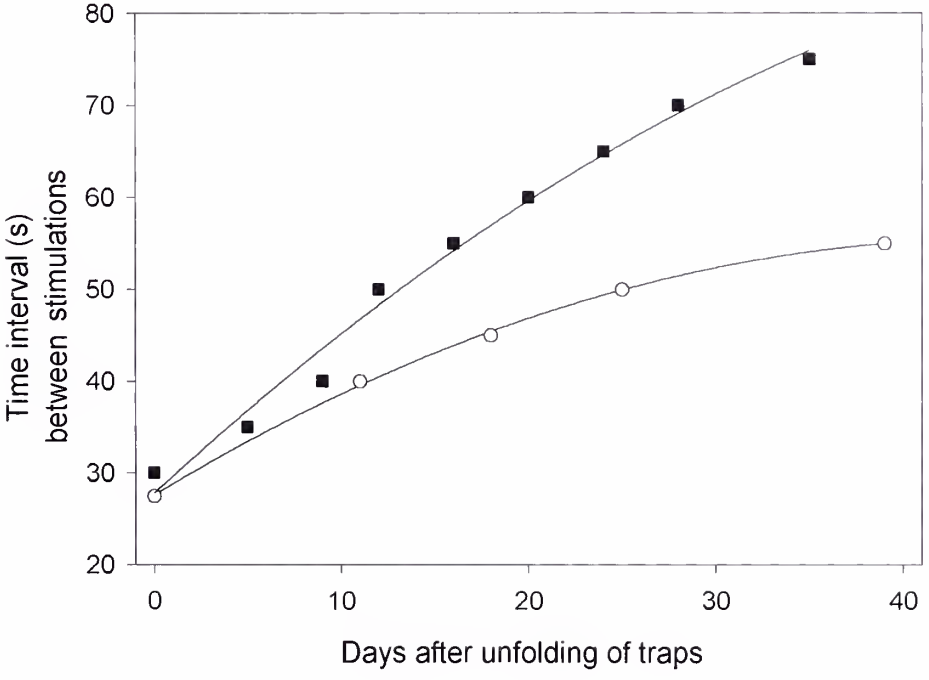

Figure 6: Seasonal shift of the time memory: All age-classes of trap leaves show higher values in mid-May (filled squares) than in late June (open circles).

observed at lower temperatures, may be attributed to the slow-down with falling temperature of an as yet unknown biochemical or biophysical process, which somehow interferes with the memory-sustaining physiological process. In addition, the temperature curve for the time memory of *D. muscipula* is sigmoidal, as confirmed by a Probit-transformation of the data (see Figure 3, Insert). Near the lower and the upper ends of the temperature curve, changes in time memory were much smaller. Beyond 30°C, practically none of the leaves closed after the 60 s time interval between first and second stimulus.

Sometimes, trap leaves close after only one stimulation of a trigger hair (Brown & Sharp 1910; Sibaoka 1966). We examined possible influences of temperature on this phenomenon. While about 14% of the traps responded after only one triggering at 25°C, the percentage declined to about 3% at 15°C (see Figure 4). This result suggests that the proposed $[Ca^{2+}]_{cyt}$ threshold value is especially low in such hypersensitive traps, and is therefore exceeded without a second Ca^{2+}-influx wave being initiated. Following this line of reasoning, one may conclude that the stimulation-induced increase in $[Ca^{2+}]_{cyt}$, has a markedly positive temperature coefficient. Alternatively, it may be imagined that the depolarisation amplitude of the first AP, and thus the resulting initial rise in $[Ca^{2+}]_{cyt}$, is outstandingly high in such trap leaves and consequently a second push of $[Ca^{2+}]_{cyt}$ is superfluous.

Effect of the Duration of Daily Irradiance

A significant difference in the time memory is attained when the daily photon fluency (mmol quanta m^{-2}) was changed. In addition to our standard setting—continuous irradiation—we included short day conditions, simulated by 8 hours of illumination and 16 hours of darkness. We apologize for not also investigating long day conditions (16 hours of illumination, 8 hours of darkness) due to the exhaustion of our plant resources. The memory time was 10 to 15 s shorter when *D. muscipula* was exposed to short day conditions (see Figure 5). We are tempted to regard this effect as an indirect one. Under short day conditions, the physiological conditions in general and photosynthetic productivity and energy supply in particular (Mayer & Hampp 1995) might be less favourable for the expression of the time memory. The importance may have been particularly enhanced since all time memory measurements were executed between 9 and 12 a.m., just after the beginning of the photoperiod at 8 a.m. This result is consistent with the interpretation that the closure movement of the *D. muscipula* trap, as in other nastic movements, is accompanied by a marked decline in ATP content in the tissues involved (Jaffe 1973; Williams & Bennett 1982; Fromm & Eschrich 1988). Meeting this energy demand might well be less problematic under continuous irradiation.

Effects from Seasonality and Leaf Age

We observed seasonal effects on the time memory of *D. muscipula.* In mid-May the time memory is clearly longer than in late June (see Figure 6). This difference includes all age classes of traps. For instance, in spring, relatively old traps exhibit a memory time of up to 75 s whereas in summer, traps of the same age show a time memory of just 50 s. Hence, the ontogenetic life cycle of the trap leaves of *D. muscipula*, which, of course, governs leaf emergence, leaf growth and leaf development, itself is subject of circannual variability (Roberts & Oosting 1958). This seasonality modulates the time memory of the Venus flytrap to a considerable extent.

The influence of the ontogenetic stage of the trap leaves on the time memory of *D. muscipula* was further confirmed by the effect of trap leaf age on the time memory. The younger the traps being investigated, the shorter was the time memory (see Figure 7). Time memory reached its maximum in the most fully developed traps; newly emerged trap leaves exhibited the shortest memory time. This result can be derived from the percentage of traps which responded to a fixed time interval (45 s) between first and second stimulation. The stimulation time interval for 50% closure shows exactly the same age effect. Evidently, mature leaves are superior to younger traps with respect to those anatomical and physiological conditions, which presumably are decisive for the manifestation of the time memory and its respective duration.

Stimulation Involving Different Trigger Hairs.

In addition to the external, i.e. environmental conditions, which have been tested above, a positional effect was also investigated, namely the stimulation of different trigger hairs and the influence thereof on the time memory. Indeed, the pattern of stimulation exerts a surprisingly strong influence on the time memory of *D. muscipula*. It makes a big difference whether the same trigger

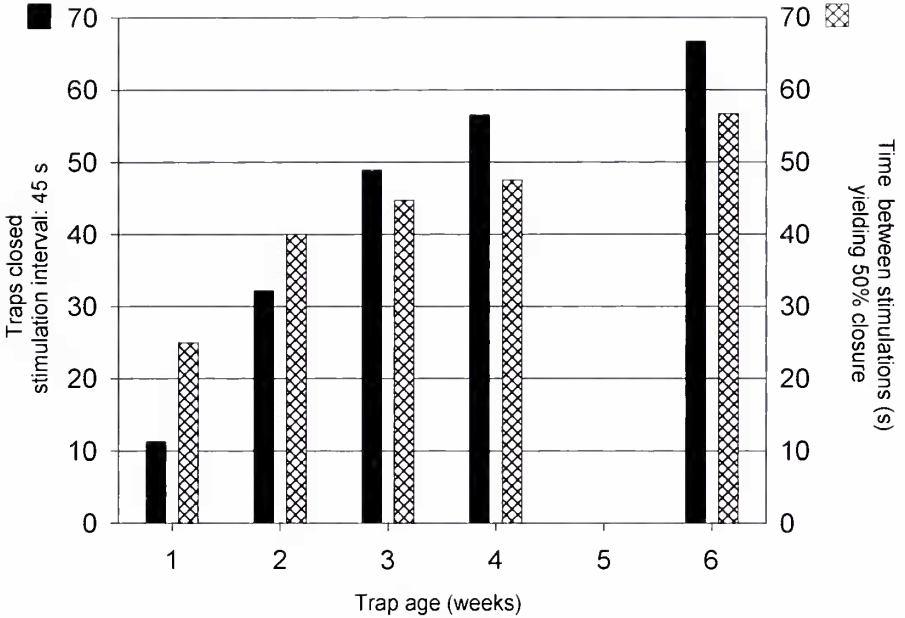

Figure 7: Influence of trap age on the time memory, expressed as the percentage of closures at 45 s time interval between stimulations (solid bars), or the number of seconds required to reach 50% closure values (crosshatched bars).

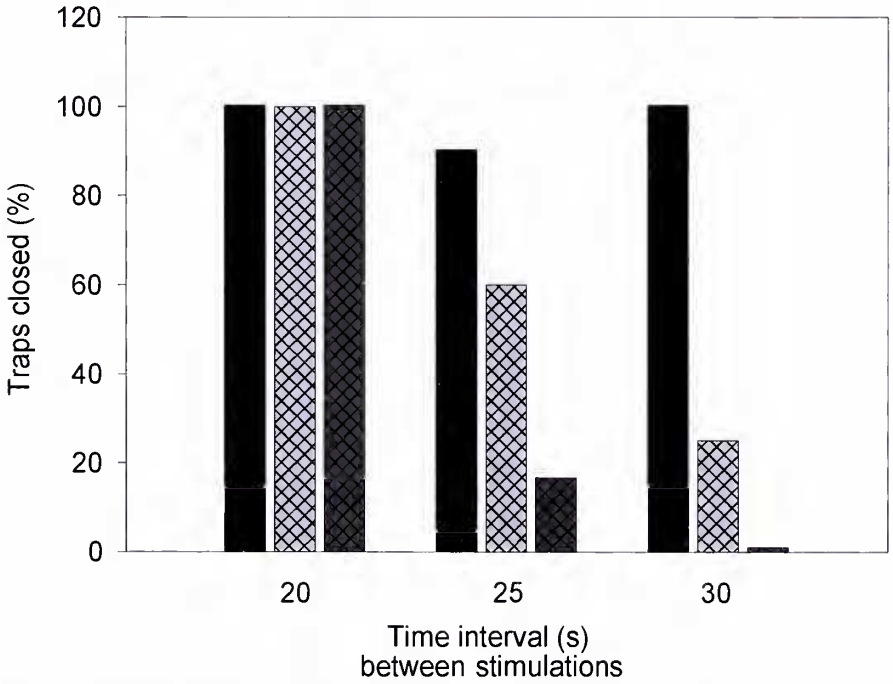

Figure 8: Position effect of the time memory. The second stimulation was executed either with the same trigger hair as before (shaded in black), or with an other hair, located either on the same lobe of the trap leaf (light grey), or on the opposite lobe (dark grey).

hair was successively stimulated twice, or two different hairs—located either on the same or on opposite lobes of the trap leaf—were stimulated one after the other. When a relatively short time-interval (20 s) between successive stimulations was applied, all traps closed, regardless of which hairs were touched or in which order (see Figure 8). However, when the interval between stimulations was extended to 25 s or even 30 s, the response of the traps depended very much on the mode of triggering. At 30 s, the percentage of traps that closed dropped almost to zero when two trigger-hairs on opposite lobes were stimulated successively. Stimulation of two different hairs, located on the same lobe also led to a decrease of memory time, but still about 20% closures were observed. An intermediate time interval, 25 s also caused a considerable and differential decrease in time memory, but the decline was less pronounced than at 30 s.

These results indicate a pronounced cooperativity of the sensitive hairs in triggering the closure movement. The differential response pattern of the time memory described above cannot be understood without assuming an exchange of information between the different trigger hairs of a trap leaf, the APs acting as the transmitter. Supposedly, the AP-signal of any first stimulation event is communicated to and perceived by all other sensitive hairs of the trap. This suggests that the time memory of *D. muscipula* cannot be attributed to any specific, exclusive cells/tissue loci, aside from the sensitive hairs, which exhibit a distinct AP-responsiveness. Fagerberg and Allain (1991) studied the dynamics of trap closure and reported that the movement is very much delocalized and all parts of the trap leaf are involved, although to differing extents. If, on the other hand, a distinct signal perception site really exists, it should not make a significant difference with respect to the resulting memory time, if the first and the second AP-signal is being perceived as a double signal from one and the same trigger hair, or if two different sensitive hairs are engaged in signal transduction.

Discussion

It has been demonstrated (see Figure 1) that while trap leaves that close incompletely apparently have a hampered closure mechanism, their time memories—although slightly shifted—are otherwise identical for total closures and all closures. This clearly shows that the time memory is not causally connected to the dynamics of the closure process. This closure-model predicts a state of mechanical bistability where both the open and the closed states are metastable to small perturbations. Assumedly, large, mature, and thus strongly curved leaves release more energy at this critical stage of movement and easily reach the state of complete closure. In contrast, smaller, more weakly curved, predominantly young leaves may get stuck before total closure is reached (Forterre *et al.* 2005).

The inverse temperature curve which characterizes the memory effect (see Figure 3) can be understood as the manifestation of a reaction (-series), which interacts antagonistically with the time memory, and which accelerates dramatically with rising temperature. If so, this reaction most likely governs the removal or redistribution of a compound, the intracellular level of which had been raised by the AP triggered by a first-stimulation event. The prime candidate for this compound in question is $[Ca^{2+}]_{cyt}$ (see Introduction). In this case, the antagonistic reaction could well comprise Ca^{2+} efflux from the cytosol, directed by Ca^{2+}-ATPases, located in the plasmalemma (and perhaps in the ER) of the cells which have been seized by the AP-wave. In fact, several ATPases have been found to exhibit extraordinarily high temperature coefficients (DeCoursey & Cherny 1998), exhibiting Q_{10}-values up to 5.3. This coincides quite well with a Q_{10} of about 4 for the decline of memory time between 20 and 30°C. In conclusion, the AP-induced enhanced level of $[Ca^{2+}]_{cyt}$ is supposed to decrease more rapidly at elevated temperatures due to a markedly enhanced activity of these Ca^{2+}-efflux pumps. As a consequence, much more rapidly $[Ca^{2+}]_{cyt}$ reaches a level, too low to be pushed beyond the critical threshold for closure by the subsequent second AP. However, the indicative value of the high Q_{10}, observed, must not be over-stressed since not only membrane transporters (DeCoursey & Cherny 1998; Zuñiga *et al.* 2004) but complex reaction sequences may exhibit above average temperature coefficients. The levelling-off of the temperature curve at >30°C and < 20°C indicate that functional integrity is rapidly lost even under not really extreme temperature conditions. In this respect, thermally induced membrane phase transitions, affecting transmembrane transport systems, e.g. the ATPases, supposedly involved in the time memory mechanism (see above), should be taken into consideration (Cooke & Burden 1990).

The interpretation of the strong position effect of stimulation (see Figure 8) is challenging and worth being discussed. If in fact the time memory is delocalized across the leaf blade of the traps,

as has been deduced above, then the propagating AP-signal, evoked by the stimulated trigger hair, assumedly starts the time memory clock everywhere across the leaf, but with a decreasing impact. Consequently, the more distant from the initially triggered hair the other hairs (stimulated thereafter) are, the weaker is the switch-on impact evoked at their respective site. For that reason, the first pulse must fade away more rapidly in the remote regions of the trap leaf than it does in the neighbourhood of the originally stimulated hair. It may be mentioned that this switch-on process does not necessarily consist of the initial rise in $[Ca^{2+}]_{cyt}$ itself (see Introduction). It could be a subsequent step in an intracellular signal chain, perhaps as a hypothetical regulator complex as hypothesized by Hodick & Sievers (1988). In any case, we find that within 30 s the second AP-pulse, evoked by a different (and remote) trigger hair, may be unable to drive the situation beyond the threshold requirement and thence trap closure. In case of a double-stimulation of the same trigger hair, after 30 s enough of the originally elevated $[Ca^{2+}]_{cyt}$ (or of any hypothetical factor) is still present to sustain the pre-closure condition of the trap. Two different trigger hairs, located next to each other, would represent an intermediate situation. The intermediate results obtained at 25 s time-lapse between stimulations support this interpretation.

The effects of irradiance (daily photon fluency), of seasonality and of trap leaf maturity (leaf age) on the time memory of *D. muscipula* are pronounced. While these results do not *per se* offer any clue for a deeper understanding of the mechanisms underlying time memory, they suggest that the time memory is deeply affected by a wide scope of physiological factors. Their variability within the natural ontogenetic band-width of trap leaves strongly radiates into the time memory mechanisms.

Conclusion

After about 130 years of observation, amazement, experimentation and interpretation, the Venus flytrap *D. muscipula* still deserves the attribute "this most remarkable of all plants" (Darwin 1875), and still, the plant is an enigma. One of the questions awaiting definite answers is the time memory. While the elucidation of calcium signalling and oscillations in $[Ca^{2+}]_{cyt}$ in guard cells and some other topics is far advanced on the methodological as well as on the theoretical scope (White 2000; Schroeder *et al.* 2001; Hetherington & Brownlee 2004), the corresponding aspects with regard to the time memory of *D. muscipula* have been completely neglected up to now. We were able to provide some new insights into the phenology of this intriguing phenomenon. They in turn may now hopefully revive and stimulate research on the molecular mechanisms of the time memory.

Acknowledgements: We are very much grateful to H. Zimmer and his team for expert nursing of the plants. This work was supported by E-Plus Mobilfunk GmbH und Co. KG (Düsseldorf, Germany) and Swisscom AG (Bern, Switzerland).

Literature Cited

Benolken, R.M., and Jacobson, S.L. 1970. Response properties of a sensory hair excised from Venus's-flytrap. J.Gen. Physiol. 56: 64-82.

Brown, W.H. 1916. The mechanism of movement on the duration of the effect of stimulation in the leaves of *Dionaea*. Amer. J. Bot. 3: 68-90.

Brown, W.H., and Sharp, L.W. 1910. The closing response in *Dionaea*. Bot. Gaz. 49: 290-302.

Burden-Sanderson, J. 1888. On the electromotive properties of the leaf of *Dionaea* in the excited and unexcited states. Phil. Trans. R. Soc. Lon. 179: 417-449.

Burden-Sanderson, J., and Page, F.J.M. 1876. On the mechanical effects and on the electrical disturbance consequent on excitation of the leaf of *Dionaea muscipula*. Proc. R. Soc. Lon. 25: 411-434.

Casser, M., Hodick, D., Buchen, B., and Sievers, A. 1985. Correlation of excitability and bipolar arrangement of endoplasmatic reticulum during the development of sensory cells in trigger hairs of *Dionaea muscipula* Ellis. Eur. J. Cell. Biol. 36 (Supp.). 12.

Cooke, D.T., and Burdon, R.S. 1990. Lipid modulation of plasma membrane-bound ATPases. Physiol. Plant. 78: 153-159.

Cosgrove, J.D., and Hedrich, R. 1991. Stretch-activated chloride, potassium and calcium channels coexisting in plasma membranes of guard cells of *Vicia faba* L. Planta. 186: 143-153.

Darwin, C. 1875. Insectivorous plants. Murray, London .
DeCoursey, T.E., and Cherny, V.V. 1998. Temperature dependence of voltage-gated H^+ Currents in human neutrophils, rat alveolar epithelial cells, and mammalian phagocytes. J. Gen. Physiol. 112: 503-522.
Fagerberg, W.R., and Allain, D. 1991. A quantitative study of tissue dynamics during closure in the traps of Venus's flytrap *Dionaea muscipula* Ellis. Am. J. Bot. 78: 647-657.
Forterre, Y., Skotheim, J.M., and Dumais, J., and Mahadevan, L. 2005. How the Venus flytrap snaps. Nature. 433: 421-425.
Fromm, J., and Eschrich, W. 1988. Transport processes in stimulated and non-stimulated leaves of *Mimosa pudica*. II. Energies and transmission of seismic stimulations. Trees. 2: 18-24.
Guttenberg, von H. 1925. Die Bewegungsmechanik des Laubblattes von *Dionaea muscipula* Ellis. Flora. 118: 165-183.
Guttenberg, von H. 1926. Die Bewegungsgewebe. In: K Linsbauer, ed, Handbuch der Pflanzenanatomie, Section 1, Part 2, Volume 5. Gebrüder Bornträger, Berlin pp 143-146.
Haberlandt, G. 1906. Sinnesorgane im Pflanzenreich. Zur Perzeption mechanischer Reize. 2 Ed. Engelmann, Leipzig: 133-143.
Harper, J.F. 2001. Dissecting calcium oscillators in plants. Trends Plant Sci. 6: 395-397.
Hetherington, A.M., and Brownlee, C. 2004. The generation of Ca^{2+} signals in plants. Ann. Rev. Plant Biol. 55: 401-427.
Hodick, D.M., and Sievers, A. 1986. The influence of Ca^{2+} on the action potential in mesophyll cells of *Dionaea muscipula* Ellis. Protoplasma. 133:83-84.
Hodick, D.M., and Sievers, A. 1988. The action potential of *Dionaea muscipula* Ellis. Planta. 174: 8-18.
Hodick, D.M., and Sievers, A. 1989. On the mechanism of trap closure of Venus flytrap (*Dionaea muscipula* Ellis). Planta. 179: 32-42 .
Jacobson, S.L. 1965. Receptor response in Venus's flytrap. J. Gen. Physiol. 49: 117-129.
Jaffe, M.J. 1973. The role of ATP in mechanically stimulated rapid closure of the Venus's-flytrap. Plant Physiol. 51: 1293-1302.
Juniper, B.E., Robins, R.J., and Joel, D.M. 1989. The carnivorous plants. Academic Press, London pp 46-129.
Malone, M. 1996. Rapid long-distance signal transmission in higher plants. Adv. Bot. Res. 22: 161-228.
Mayer, W.-E., and Hampp, R. 1995. Movement of pulvinated leaves. Progr. Bot. 56: 236-262.
Roberts, P.R., and Oosting, H.J. 1958. Responses of Venus fly trap (*Dionaea muscipula*) to factors involved in its endemism. Ecol. Monogr. 28: 193-218.
Schroeder, J.L., Allen, G.J., Hugouvieux, V., Kwak, J.M., and Waner, D. 2001. Guard cell signal transduction. Ann. Rev. Plant Physiol. Plant. Mol. Biol. 52: 627-658.
Sibaoka, T. 1966. Action potentials in plant organs. Symp. Soc. Exp. Biol. 20: 49-73 .
Sibaoka, T. 1991. Rapid plant movements triggered by action potentials. Bot. Mag. Tokyo. 104: 73-95.
Stuhlmann, O. 1948. A physical analysis of the opening and closing movements of the lobes of Venus's fly-trap. Bull. Torrey. Bot. Club. 75: 22-44.
Trebacz, K., and Busch, M.B., Hejnowicz Z., Sievers A. 1996. Cyclopiazonic acid disturbs the regulation of cytosolic calcium when repetitive action potentials are evoked in *Dionaea* traps. Planta. 198: 623-626.
Volkov, A.G., Shvetsova, T., and Markin, V.S. 2002. Waves of excitation and action potentials in Green Plants. J. Biophys. 82: 218a.
Wayne, R. 1994. The excitability of plant cells: With a special emphasis on *Characean* internodal cells. Bot.Rev. 60: 265-367.
White, P.J. 2000. Calcium channels in higher plants. Ann. Bot. 81: 173-183.
Williams, S.E. 1973. The memory of the Venus' flytrap. Carniv. Pl. Newslett. 2: 23-25.
Williams, S.E., and Bennett, A.B. 1982. Leaf closure in the Venus flytrap: An acid growth response. Science. 218: 1120-1122.
Williams, S.E., and Mozingo, H.N. 1971. The fine structure of the trigger hair in Venus's flytrap. Amer. J. Bot. 58: 532-539.
Williams, S.E., and Pickard, B.G. 1980. The role of action potentials in the control of capture movements of *Drosera* and *Dionaea*. In: F. Skoog ed., Plant growth substances. Springer, Berlin, Heidelberg, New York pp 470-480.